Brain, Behavior, and Immunity xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Effect of long-term sleep restriction and subsequent recovery sleep on the diurnal rhythms of white blood cell subpopulations

Julie Lasselin [a,b], Javaid-ur Rehman [a], Torbjorn Åkerstedt [a,b], Mats Lekander [a,b,c], John Axelsson [a,b,c,*]

[a] Department of Clinical Neuroscience, Karolinska Institutet, Nobels väg 9, 171 65 Solna, Stockholm, Sweden
[b] Stress Research Institute, Stockholm University, Frescati Hagväg 16A, 106 91 Stockholm, Sweden
[c] Osher Center for Integrative Medicine, Karolinska Institutet, Scheeles väg 1, 171 65 Solna, Stockholm, Sweden

ARTICLE INFO

Article history:
Received 9 July 2014
Received in revised form 5 October 2014
Accepted 6 October 2014
Available online xxxx

Keywords:
Sleep restriction
Sleep deprivation
Recovery sleep
Diurnal rhythms
Circadian rhythms
Leukocytes
White blood cells
Monocytes
Lymphocytes
Neutrophils

ABSTRACT

While acute modifications of sleep duration induces a wide array of immune function alterations, less is known of how longer periods with insufficient sleep affect immune functions and how they return to normal once recovery sleep is obtained. The purpose of the present study was to investigate the effects of five days of restricted sleep and a subsequent 7-day period of sleep recovery on white blood cell (WBC) subpopulation count and diurnal rhythms. Nine healthy males participated in a sleep protocol consisting of two baseline days (8 h of sleep/night), five nights with restricted sleep (4 h of sleep/night) and seven days of recovery sleep (8 h of sleep/night). During nine of these days, blood was drawn hourly during nighttime end every third hour during daytime, and differential WBC count was analyzed. Gradual increase across the days of sleep restriction was observed for total WBC ($p < .001$), monocytes ($p < .001$), neutrophils ($p < .001$) and lymphocytes ($p < .05$). Subsequent recovery sleep resulted in a gradual decrease in monocytes ($p < .001$) and lymphocytes ($p = .001$), but not in neutrophils that remained elevated over baseline level at the end of the 7-day recovery period. These effects were associated with altered diurnal rhythms of total WBC and neutrophils, restricted sleep being associated with higher levels during the night and at awakening, resulting in a flattening of the rhythm. The diurnal alterations were reversed when recovery sleep was allowed, although the amplitude of total WBC, neutrophils and monocytes was increased at the end of the recovery period in comparison to baseline. Altogether, these data show that long-term sleep restriction leads to a gradual increase of circulating WBC subpopulations and alterations of the respective diurnal rhythms. Although some of the effects caused by five days of restricted sleep were restored within the first days of recovery, some parameters were not back to baseline even after a period of seven recovery days.

1. Introduction

The bidirectional association that exists between sleep and the immune system is well-acknowledged, with sleep regulating immune function as well as it being modulated by the immune system (e.g., during infection) (Bryant et al., 2004; Majde and Krueger, 2005; Mullington et al., 2010). Both lack of sleep and alterations in immune function are associated with increased risk of developing several physical and psychiatric diseases, including obesity, type 2 diabetes, and major depression (Shoelson et al., 2007; Miller et al., 2009; Spiegel et al., 2009; Roberts and Duong, 2014). In addition, insufficient sleep is associated with a higher susceptibility to infections (Cohen et al., 2009). A better understanding of how insufficient sleep affects immune functions is vital if we are to understand the etiology of sleep-immune related diseases.

Variation in sleep duration, either experimentally manipulated or in studies of natural variation, is associated with alterations of immune function, reflected by modulations of both immune cell number and activity (Dinges et al., 1995; Irwin, 2002). For example, decreased activity of natural killer (NK) lymphocytes has been demonstrated after an experimental restriction of sleep duration to 5 h in healthy men (Irwin et al., 1996) and after natural short sleep (i.e., <7 h of sleep) (Fondell et al., 2011). In addition, acute deprivation of sleep, either total or partial, induces an increase in the number of circulating white blood cells (WBC), in particular granulocytes and neutrophils, in healthy men (Heiser et al., 2000; Boudjeltia et al., 2008; Faraut et al., 2011) or in a group of healthy men and women (Dinges et al., 1994). Altogether, these results

* Corresponding author at: Department of Clinical Neuroscience, Karolinska Institutet, Nobels väg 9, 171 65 Solna, Stockholm, Sweden. Tel.: +46 (0)8 524 824 61.
E-mail address: john.axelsson@ki.se (J. Axelsson).

http://dx.doi.org/10.1016/j.bbi.2014.10.004
0889-1591/

indicate that insufficient sleep alters both immune function and the redistribution of leukocytes. Nevertheless, only a few studies have explored the effects of a prolonged period of insufficient sleep, which is common in today's modern society (CDC, 2014), on immune function. These studies support the notion that longer period with insufficient sleep (i.e., 5–10 days) leads to an increase of systemic levels of inflammatory markers (Meier-Ewert et al., 2004; Vgontzas et al., 2004; van Leeuwen et al., 2009). In addition, a recent study suggests a shift of T helper (Th) activity toward a type 2 cytokine profile at the expense of the type 1 profile, reflected by a decrease in the IL2/IL4 ratio, after five days of sleep restriction in healthy men (Axelsson et al., 2013), which is similar to the effects of acute total sleep deprivation (Dimitrov et al., 2004; Lange et al., 2006). Although these results suggest both acute and sustained periods with insufficient sleep to affect immune function, there is still a poor understanding of possible dynamic changes occurring over periods with insufficient sleep. In addition, to improve the interpretation of how insufficient sleep affects immune function, there is a need to investigate how it affects immune cell subpopulations. Furthermore, the question of how immune functions are restored during recovery sleep is poorly explored. One study indicates that two nights of recovery is too short after a five-day period with restricted sleep to restore immune function (van Leeuwen et al., 2009). Notably, this study, as well as many other studies in the field, suffers from infrequent blood sampling (typically only a morning sample) which limits the possibility to describe diurnal changes of immune function.

It is likely that the modulation in immunological markers and immune cell numbers during insufficient sleep are associated with alterations in circadian rhythms. The number of WBC indeed follows specific circadian rhythms, which in turn are strongly modulated under acute total sleep deprivation (Born et al., 1997; Lange et al., 2010). For example, one recent study indicates that the amplitude in the rhythm of granulocytes is reduced after a prolonged period of wakefulness in men (Ackermann et al., 2012). Nevertheless, to the best of our knowledge, the effect of a modest but sustained sleep restriction, a relevant model for suboptimal sleep patterns in today's society (CDC, 2014), on diurnal rhythms of WBC has never been investigated.

The objective of the present study was therefore to assess the changes in leukocyte subpopulations count and diurnal rhythms that develop over five days of restricted sleep, as well as the potential restoring effect of a subsequent 7-day period of sleep recovery.

2. Subjects and methods

2.1. Participants

Nine healthy men subjects (age: 22–27 years) participated in the study. The exclusion criteria were any physiological or psychiatric pathology, medication, obesity (body mass index range of included subjects: 21–26 kg/m^2), smoking, binge drinking or excessive caffeine use (more than 3 cups per day). All subjects had regular sleep habits and a habitual sleep need between 7 and 8.5 h. The study was approved by the regional ethical review board in Stockholm, Sweden. All participants signed a written informed consent after a complete explanation of the study.

2.2. Study protocol

The study protocol has been previously described in details, where we analyzed the *in vitro* Th1/Th2-balance after five days with restricted sleep (blood samples taken during baseline and day 5 with restricted sleep were stimulated *in vitro*) (Axelsson et al., 2013; Lekander et al., 2013), and sleep homeostasis during sleep restriction (Akerstedt et al., 2009). Briefly, subjects were asked to follow a strict sleep schedule with 8 h of sleep starting two weeks before the beginning of the protocol. Then, subjects spent one habituation day (8 h of sleep) in the sleep laboratory at the National Institute of Psychosocial Medicine, Stockholm, Sweden. The protocol was composed of twelve laboratory days and nights: one habituation day (8 h of sleep: 23.00 h–07.00 h), two baseline days (B1 and B2, 8 h of sleep: 23.00–07.00 h), five days with restricted sleep (RS1 to RS5, 4 h of sleep: 03.00–07.00 h), three days of recovery sleep (R1 to R3, 8 h of sleep: 23.00–07.00 h) and a last day of recovery sleep after that subjects spent three nights at home following the sleep pattern of 8 h of sleep (R7, 8 h of sleep: 23.00–07.00 h). Subjects were asked to refrain from hard physical activity at least two days before coming to the laboratory and throughout all the protocol. In order to parallel normal behavior, subjects were outdoors at least twice each day, once in the morning and once in the afternoon, under surveillance of a research assistant. The participants were also lying down in bed from 22.00 h to 08.00 h in all conditions, and staying in a supine position 30 min prior blood sampling during daytime samples (11.00 h to 20.00 h). The sleep data have been extensively described earlier (Akerstedt et al., 2009).

2.3. Blood samples and measurement of white blood cell subpopulations

Blood samples were taken every hour between 23.00 h and 08.00 h and every third hour between 08.00 h and 23.00 h (i.e., 11.00 h, 14.00 h, 17.00 h and 20.00 h). The samples included for each day in the analyses were those from normal bedtime (23.00 h) to the end of the next day (20.00 h). Blood samples were obtained during habituation sleep (but not analyzed), during the two baseline days (i.e., B1 and B2), during three days with restricted sleep (i.e., RS1, RS2 and RS5), and during the four laboratory days of recovery sleep (i.e., R1, R2, R3 and R7). Sampling during the night was conducted from an adjacent room to minimize disturbances of sleep (analyses between sleep during nights with and without blood sampling showed that blood drawing reduced sleep efficiency of about 1–1.5%). The intravenous (IV) catheter was inserted two hours before sampling was started. All samples included, 640 ml of blood was drawn from each participant (160 samples per individual of 4 ml each) over the 15 day protocol (from B1 to R7).

WBC were analyzed from EDTA-samples, which were assayed for blood counts with leukocyte differentials within 4 h of sampling by the Karolinska University Laboratory, Solna, Stockholm, Sweden. The WBC count and differential WBC count (which includes lymphocytes, monocytes, neutrophils and eosinophils) were analyzed by flow-cytometry (ADVIA 120/2120, Siemens).

2.4. Statistical analyses

The measurements during the two baseline days, B1 and B2, were combined in order to form a single baseline day (B). The effect of sleep restriction and subsequent recovery sleep on average number of WBC subpopulations was evaluated by linear mixed regression analyses using SPSS 22 (IBM). The fixed effect was either the number of days of sleep restriction (i.e., from B = 0 to RS5 = 5) or the number of days of sleep recovery (i.e., from RS5 = 0 to R7 = 7). Random effects were time of day and participants. Specific differences between days were then tested by post hoc LSD tests when appropriate. In addition, persistent effects after the 7-day period of sleep recovery was evaluated by mixed regression analyses with B *vs* RS7 as fixed effect, and time of day and participants as random effect.

In order to assess the diurnal rhythms of the WBC subpopulations and its changes over the days with sleep restriction and sub-

Please cite this article in press as: Lasselin, J., et al. Effect of long-term sleep restriction and subsequent recovery sleep on the diurnal rhythms of white blood cell subpopulations. Brain Behav. Immun. (2014), http://dx.doi.org/10.1016/j.bbi.2014.10.004

sequent recovery sleep, we applied cosinor analysis. This analysis allows determination of the cosinor curve of diurnal variation in WBC subpopulations, for each subject and each day, by extracting the cosinor values from a linear regression analysis with SINtime [=sin(2 * π * (time + 1)/24)] and COStime [=cos(2 * π * (time + 1)/24)] as independent variables. The modification in nocturnal and day variation of WBC subpopulations after sleep restriction or recovery was assessed by performing similar mixed regression analyses as described above on individual cosinor values, but by adding the interaction with either "night-time" (i.e., from 23.00 h to 07.00 h) or "daytime" (i.e., from 07.00 h to 20.00 h) as fixed effect (and by removing time of day as random effect). Planned analyses were performed between respective time points of baseline (B) *vs* the last day of restriction sleep (RS5) and the last day of restriction sleep (RS5) *vs* the last day of recovery sleep (R7). In addition, the average amplitude [=maximum – mesor (diurnal mean)] of the individual diurnal curves of WBC subpopulations at the end of the recovery period (R7) was compared to baseline (B). All probabilities were two-sided with the degree of significance set at $p < .05$.

3. Results

3.1. Effect of sleep restriction and subsequent recovery sleep on WBC subpopulations

Both sleep restriction and recovery sleep significantly affected WBC subpopulations in the circulation. Numbers of total WBC, monocytes, neutrophils and lymphocytes all increased linearly across days with sleep restriction ($p < .001$ for all except lymphocytes, $p < .05$) (Fig. 1A–D**)**. Post hoc analyses indicated that the increase for WBC and neutrophil numbers was significant already on the first day of sleep restriction, indicating an acute effect of the first night of sleep restriction (RS1, RS2, RS5: $p < .001$ *vs* B). The increase in monocytes became significant on the second day of sleep restriction (RS2 *vs* B: $p = .023$ and RS5 *vs* B: $p < .001$), while lymphocyte numbers were significantly higher first after five days of sleep restriction ($p < .05$).

Recovery sleep after five days of restricted sleep resulted in a significant gradual reduction of monocyte and lymphocyte numbers across days (p<.001 and $p = .001$, respectively) (Fig. 1C and **D**). The reductions appeared already on the first day of sleep recovery, with a slight rebound on the third day for lymphocyte numbers (monocytes, R1 to R7 *vs* B, $p < .01$; lymphocytes, R1, R2, R7 *vs* B, $p \leqslant .001$, R3 *vs* R2, $p < .05$ and R3 *vs* R7, $p < .01$). At the end of the 7-day recovery period, the number of lymphocytes was lower than the baseline levels (R7 *vs* B, $p < .05$). In contrast to these results, the number of total WBC and neutrophils did not change significantly over the days of sleep recovery (respectively, $p = .099$ and $p = .769$) (Fig. 1A and **B**). Accordingly, neutrophil numbers were still significantly increased even after seven recovery days as compared to baseline (R7 *vs* B, $p < .001$).

No significant effect of either restriction or sleep recovery was found for the average number of eosinophils (sleep restriction: $p = .455$; sleep recovery; $p = .218$) (Fig. 1E).

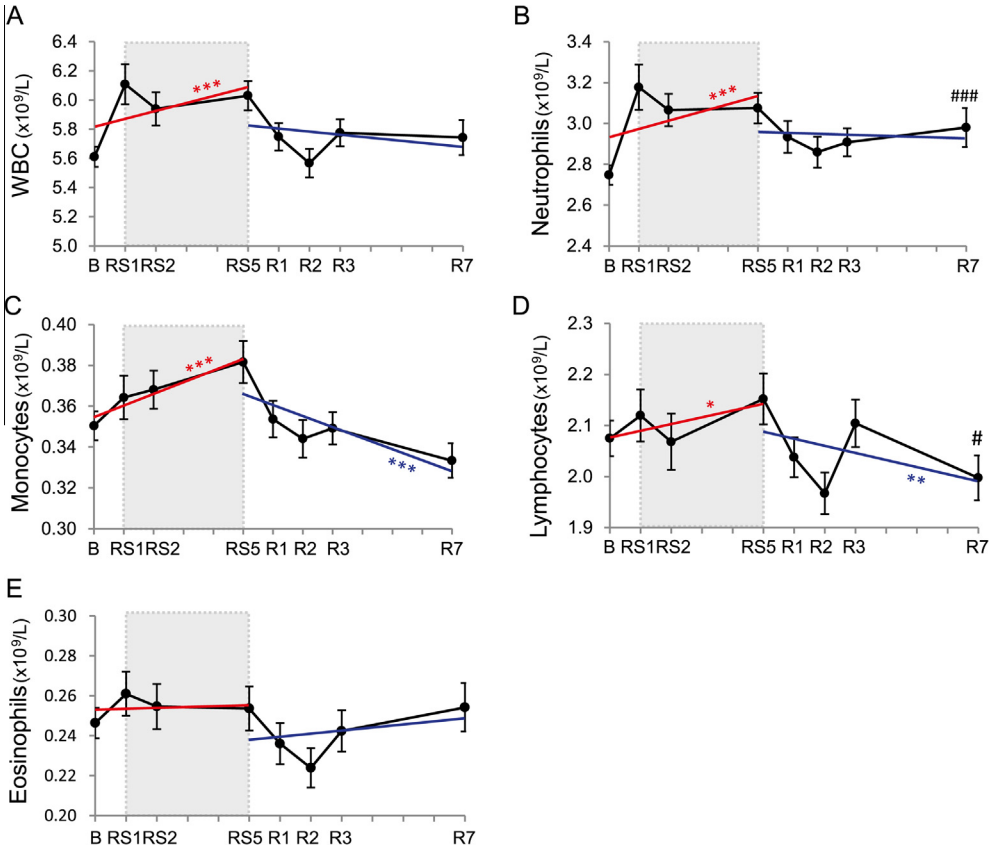

Fig. 1. Mean levels of WBC subpopulations across days of sleep restriction and subsequent recovery sleep. Linear mixed regression analyses were performed to assess the effect of sleep restriction or sleep recovery on WBC subpopulations. The regression lines represent the fixed effect of either days of sleep restriction (i.e., B = 0, RS1 = 1, RS2 = 2, RS5 = 5; red line) or days of sleep recovery (i.e., RS5 = 0, R1 = 1, R2 = 2, R3 = 3, R7 = 7; blue line). Random effects are time of day and participants. $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$. Persistent effects after the 7-day period of sleep recovery was evaluated by mixed regression analyses with B *vs* R7 as fixed effect, and time of day and participants as random effect. $^{\#}p < .05$ *vs* B; $^{\#\#\#}p < .001$ *vs* B. The gray section represents the sleep restriction period. *Abbreviations*: B = Baseline; RS = Restricted Sleep; R = Recovery sleep; WBC = total White Blood Cells. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Please cite this article in press as: Lasselin, J., et al. Effect of long-term sleep restriction and subsequent recovery sleep on the diurnal rhythms of white blood cell subpopulations. Brain Behav. Immun. (2014), http://dx.doi.org/10.1016/j.bbi.2014.10.004

3.2. Diurnal rhythms of WBC subpopulations after sleep restriction and subsequent recovery sleep

Separate analyses were made for changes during night-time (normal sleep, 23.00 h–07.00 h) and daytime (normal waking, 07.00 h–20.00 h), respectively, to elucidate where the diurnal changes were found (Fig. 2). Overall, the diurnal rhythms were not drastically altered by sleep restriction, although levels of WBC, neutrophils and eosinophils were increased during the night-time (from 00.00 h for WBC and neutrophils, and from 04.00 h for eosinophils) and morning hours (interaction effects, $p < .01$; WBC and neutrophils, B < RS5: at 00.00 h, $p < .05$, between 01.00 h and 03.00 h, $p < .01$, between 04.00 h and 08.00 h, $p < .001$, at 11.00 h, $p < .01$; eosinophils, B < RS5: between 04.00 h and 05.00 h, $p < .05$, between 06.00 h and 08.00 h, $p < .01$, and at 11.00 h, $p < .05$), which resulted in a flattening of the rhythm for WBC and neutrophils as compared to baseline, see Fig. 2A and C. The flattening of the diurnal rhythms of WBC and neutrophils were

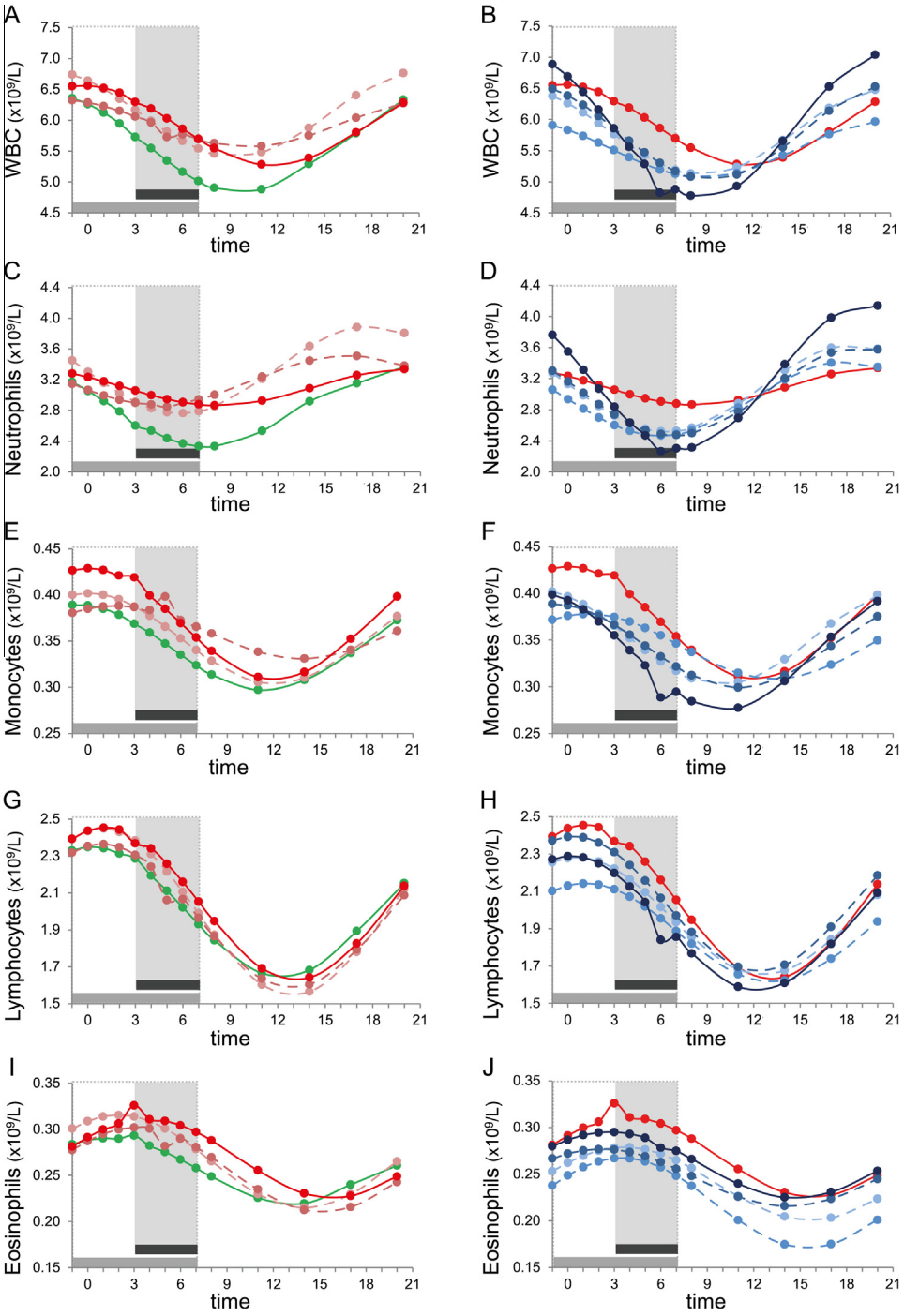

Fig. 2. Diurnal variations of WBC subpopulations across days of sleep restriction (left panels) and subsequent recovery sleep (right panels). Diurnal variations curves were obtained from cosinor analysis (see 2.4). Lefts and right panels represent cosinor curves of WBC subpopulations across the days of sleep restriction and recovery sleep, respectively. Sleep during the 8-h nights (i.e., baseline and recovery sleep) Sleep during sleep restriction. Left panels: B RS1 RS2 RS5 Right panels: RS5 R1 R2 R3 R7 *Abbreviations*: B = Baseline; RS = Restricted Sleep; R = Recovery sleep; WBC = total White Blood Cells.

Please cite this article in press as: Lasselin, J., et al. Effect of long-term sleep restriction and subsequent recovery sleep on the diurnal rhythms of white blood cell subpopulations. Brain Behav. Immun. (2014), http://dx.doi.org/10.1016/j.bbi.2014.10.004

reversed during recovery sleep (Fig. 2B and D), again showing decrease during the night-time, with lower morning levels and a steeper rise during daytime (interaction effects: $p < .001$; WBC: RS5 > R7 between 04.00 h and 08.00 h, $p < .01$ and RS5 < R7 between 17.00 h and 20.00 h, $p = .001$; neutrophils: RS5 > R7 between 05.00 h and 08.00 h, $p < .05$ and RS5 < R7 between 17.00 h and 20.00 h, $p \leqslant .001$). Similar, but more subtle, effect of sleep recovery was observed for diurnal rhythms of monocytes (daytime × sleep recovery: $p < .05$; RS5 > R7 at 01.00 h, $p < .05$; between 02.00 h and 08.00 h, $p < .01$) (Fig. 2**F**). The amplitudes of the diurnal variation of WBC, monocytes and neutrophils were significantly higher at the end of the recovery period (R7) in comparison to baseline (WBC and neutrophils, $p < .01$; monocytes, $p < .05$). No significant effect over the days of sleep restriction and sleep recovery was found for the diurnal variation of lymphocytes, indicating that the main effects found (an increase during sleep restriction and reduction during recovery) were related to an overall increase rather at a particular time point (Fig. 2G and H).

4. Discussion

Our results support the notion that restricting sleep to 4 h per night for five subsequent nights leads to gradually increased levels of subsets of WBC in the circulation. This extends to previous studies showing increased levels of WBC subpopulations, in particular neutrophils, after acute sleep deprivation or short-term sleep restriction (Dinges et al., 1995; Heiser et al., 2000; Boudjeltia et al., 2008; Faraut et al., 2011). Importantly, our study is the first to show the kinetics over the days of restricting sleep, and indicate that total WBC and neutrophils are acutely increased (appearing after a single day of restricted sleep) while the increase in monocytes and lymphocytes surfaces gradually across five days of restricted sleep. The delayed and gradual effect on monocytes or lymphocytes could partly explain the difficulty in detecting differences when studying the modifications after one to three nights *vs* before sleep restriction (Boudjeltia et al., 2008; Faraut et al., 2011), while the effect was apparent, at least on some lymphocytes subsets, after five nights of sleep restriction (van Leeuwen et al., 2009).

The study also supports the notion that recovery sleep gradually restores the number of WBC back to normal, in particular for lymphocytes and monocytes numbers, which started to decrease from the first day of sleep recovery. However, the number of neutrophils, and consequently the total number of WBC, did not show reduced numbers during recovery sleep, the numbers of neutrophils remaining higher than baseline levels at the end of the seven-day recovery period. There is a lack of earlier studies on effect of recovery from sleep restriction on immune function, particularly of sustained periods with restricted sleep. The few studies are in line with results from the present study, with a significant return to baseline of B lymphocytes but a remaining increase of neutrophils after one or two nights of sleep recovery (van Leeuwen et al., 2009; Faraut et al., 2011). However, our study reveals additional information on the effect of recovery sleep since it provides a detailed account over the days of a longer period of recovery sleep (i.e. 7 days of recovery sleep) on WBC and their subsets. Overall our study points out the importance of enclosing multiple days of data collection when analyzing immune consequences of sleep restriction and recovery, since progressive modifications occur across days.

The effect of sleep restriction and subsequent sleep recovery for the levels of WBC subpopulations were partly related to shifts of the diurnal rhythms of these cells. In particular, the diurnal rhythms of WBC and neutrophils were flattened across days with restricted sleep, with higher levels at awakening and slower increase during the day. The present design restricting sleep by delaying bedtime with 4 h (bedtimes being 03.00 h rather than 23.00 h) strongly affected the redistribution of white blood cells during night hours so that the normal progression and reduction of WBC and neutrophils occurring during sleep were delayed, and resulted in higher levels of these immune cells in the circulation, particularly during night hours and at wake time. Overall, these results indicate that sleep restriction affects the diurnal rhythms of WBC subpopulations, supporting a redistribution of these cells to other tissues, as previously suggested (Born et al., 1997). In addition, our results illustrate the importance of frequent sampling since changes might not be apparent if they occur at another time point than where the effects exist.

Recovery sleep after sleep restriction had overall reversal effects than restriction sleep on diurnal variations of WBC subpopulations, but with some signs that not all levels were back to baseline even after 7 days of recovery. Indeed, while diurnal variations seemed to return to baseline over the first days of sleep recovery, they were then exaggerated between the third and the seventh day. Diurnal variation was therefore more pronounced at the end of the seven-day period of recovery sleep than at baseline day, notably reflected by higher amplitude of the diurnal curves. These results further highlight the importance of studying longer periods of recovery sleep after sleep restriction and suggest that adaptive mechanisms that establish after changes in sleep quantity need time before stabilizing. Although the observed effects at the seventh recovery day could reflect a consequence of returning to the laboratory after three nights spent at home, neither objective or subjective sleep variables during the seventh day of sleep recovery differed from baseline nor from third days of sleep recovery, argue against this possibility (Akerstedt et al., 2009). In short, the participants subjective ratings of sleep quality was back to baseline after the second recovery night and objectively after the third recovery night.

A limitation with the present study is the restriction of subjects' gender to men. Although an inclusion of women would have improved generalizability, it would have reduced the power without extensively increasing the number of participants. A particular complication with long-term studies of women is the need to account for how the menstrual cycle affects both sleep and immune function. Further studies in women would however be highly relevant since women are more likely to suffer from sleep disturbances such as insomnia and restless legs syndrome (Manconi et al., 2012; Green et al., 2014). Another limitation is the small sample size of nine individuals. On the other hand, the analyses were based on 14 samples per individual and day on 9 different days giving a good power for the main analyses, i.e. changes across days and the pattern of the diurnal rhythms. In addition, the present study explored the variations in number of WBC subpopulations (e.g., overall population of monocytes, lymphocytes, and neutrophils), and no functional indices. Previous studies suggest that the effect of sleep restriction differs depending on the immune cell subtypes and function (Fondell et al., 2011; Lange et al., 2006; Axelsson et al., 2013). Therefore, the present findings would merit to be confirmed and extended by assessing subpopulations of lymphocytes (e.g., B-, NK- and T-subtypes) and monocytes (e.g., M1 and M2), as well as the population diversity of neutrophils, to get better index of immune cell changes.

The results from the present study may be clinically relevant, since the modifications in number of circulating WBC and their diurnal rhythms over the days of sleep restriction suggest changes in immune system functioning. Recent animal studies support this notion by showing that the diurnal rhythm of immune cells affects the susceptibility to pathogens (Bellet et al., 2013; Druzd et al., 2014). The recurrence or chronicity of periods of insufficient sleep might therefore contribute to increase the risk to develop several diseases that are associated with immune alterations, such as

Please cite this article in press as: Lasselin, J., et al. Effect of long-term sleep restriction and subsequent recovery sleep on the diurnal rhythms of white blood cell subpopulations. Brain Behav. Immun. (2014), http://dx.doi.org/10.1016/j.bbi.2014.10.004

cardiovascular diseases, type 2 diabetes or depression (Tsujimura et al., 2009; Kerkhofs and Boudjeltia, 2012; Capuron and Miller, 2011; Lasselin and Capuron, 2014). The present results may also be clinically relevant for groups who are often exposed to periods with insufficient sleep, such as shift workers. There is some support for shift workers to have higher WBC count (Nishitani and Sakakibara, 2007), although it was not associated with alterations in immune system functions (van Mark et al., 2010; Copertaro et al., 2011). However, shift-work studies are limited to a single blood sample and it is possible that alterations of the diurnal rhythmicity might have been missed.

To conclude, the present study provides the first evidence for a gradual increase of WBC subpopulations in circulation during sustained sleep restriction. This increase during sleep restriction was associated with distinctly different diurnal alterations for the respective subpopulations. Sleep restriction resulted in that lymphocytes kept a stable diurnal rhythm with a general increase, but in a flattening of the diurnal rhythms of WBC and neutrophils. This highlights the importance of studying long-term effect of sleep restriction and diurnal patterns by using frequent blood samples over extended periods. Importantly, although recovery sleep seemed to restore most effects of restricting sleep, all numbers and diurnal patterns were not back to baseline, even after seven days of recovery sleep. Further studies will have to investigate more detailed changes of subsets and whether such changes relate to changed immune functions.

Conflict of interest

All authors declare no conflict of interest.

Acknowledgments

This study was funded by the Swedish Tercentary Fund and the Swedish fund for working life. We thank PhD Paolo D'Onfrio for help during the data collection, and the participants.

References

Ackermann, K., Revell, V.L., Lao, O., Rombouts, E.J., Skene, D.J., Kayser, M., 2012. Diurnal rhythms in blood cell populations and the effect of acute sleep deprivation in healthy young men. Sleep 35, 933–940. http://dx.doi.org/10.5665/sleep.1954.

Akerstedt, T., Kecklund, G., Ingre, M., Lekander, M., Axelsson, J., 2009. Sleep homeostasis during repeated sleep restriction and recovery: support from EEG dynamics. Sleep 32, 217–222.

Axelsson, J., Rehman, J.U., Akerstedt, T., Ekman, R., Miller, G.E., Hoglund, C.O., Lekander, M., 2013. Effects of sustained sleep restriction on mitogen-stimulated cytokines, chemokines and T helper 1/T helper 2 balance in humans. PloS One 8, e82291. http://dx.doi.org/10.1371/journal.pone.0082291.

Bellet, M.M., Deriu, E., Liu, J.Z., Grimaldi, B., Blaschitz, C., Zeller, M., Edwards, R.A., Sahar, S., Dandekar, S., Baldi, P., George, M.D., Raffatellu, M., Sassone-Corsi, P., 2013. Circadian clock regulates the host response to Salmonella. Proc. Natl. Acad. Sci. USA 110, 9897–9902. http://dx.doi.org/10.1073/pnas.1120636110.

Born, J., Lange, T., Hansen, K., Molle, M., Fehm, H.L., 1997. Effects of sleep and circadian rhythm on human circulating immune cells. J. Immunol. 158, 4454–4464.

Boudjeltia, K.Z., Faraut, B., Stenuit, P., Esposito, M.J., Dyzma, M., Brohee, D., Ducobu, J., Vanhaeverbeek, M., Kerkhofs, M., 2008. Sleep restriction increases white blood cells, mainly neutrophil count, in young healthy men: a pilot study. Vasc. Health Risk Manage. 4, 1467–1470.

Bryant, P.A., Trinder, J., Curtis, N., 2004. Sick and tired: does sleep have a vital role in the immune system? Nat. Rev.: Immunol. 4, 457–467. http://dx.doi.org/10.1038/nri1369.

Capuron, L., Miller, A.H., 2011. Immune system to brain signaling: neuropsychopharmacological implications. Pharmacol. Therap. 130, 226–238. http://dx.doi.org/10.1016/j.pharmthera.2011.01.014.

CDC, 2014. Insufficient Sleep Is a Public Health Epidemic. Centers for Disease Control and Prevention Features. <http://www.cdc.gov/features/dssleep/> (accessed August 2014).

Cohen, S., Doyle, W.J., Alper, C.M., Janicki-Deverts, D., Turner, R.B., 2009. Sleep habits and susceptibility to the common cold. Arch. Internal Med. 169, 62–67. http://dx.doi.org/10.1001/archinternmed.2008.505.

Copertaro, A., Bracci, M., Gesuita, R., Carle, F., Amati, M., Baldassari, M., Mocchegiani, E., Santarelli, L., 2011. Influence of shift-work on selected immune variables in nurses. Ind. Health 49, 597–604.

Dimitrov, S., Lange, T., Tieken, S., Fehm, H.L., Born, J., 2004. Sleep associated regulation of T helper 1/T helper 2 cytokine balance in humans. Brain, Behav., Immunity 18, 341–348. http://dx.doi.org/10.1016/j.bbi.2003.08.004.

Dinges, D.F., Douglas, S.D., Zaugg, L., Campbell, D.E., McMann, J.M., Whitehouse, W.G., Orne, E.C., Kapoor, S.C., Icaza, E., Orne, M.T., 1994. Leukocytosis and natural killer cell function parallel neurobehavioral fatigue induced by 64 hours of sleep deprivation. J. Clin. Invest. 93, 1930–1939. http://dx.doi.org/10.1172/JCI117184.

Dinges, D.F., Douglas, S.D., Hamarman, S., Zaugg, L., Kapoor, S., 1995. Sleep deprivation and human immune function. Adv. Neuroimmunol. 5, 97–110.

Druzd, D., de Juan, A., Scheiermann, C., 2014. Circadian rhythms in leukocyte trafficking. Seminars Immunopathol. 36, 149–162. http://dx.doi.org/10.1007/s00281-013-0414-4.

Faraut, B., Boudjeltia, K.Z., Dyzma, M., Rousseau, A., David, E., Stenuit, P., Franck, T., Van Antwerpen, P., Vanhaeverbeek, M., Kerkhofs, M., 2011. Benefits of napping and an extended duration of recovery sleep on alertness and immune cells after acute sleep restriction. Brain, Behav., Immunity 25, 16–24. http://dx.doi.org/10.1016/j.bbi.2010.08.001.

Fondell, E., Axelsson, J., Franck, K., Ploner, A., Lekander, M., Balter, K., Gaines, H., 2011. Short natural sleep is associated with higher T cell and lower NK cell activities. Brain, Behav., Immunity 25, 1367–1375. http://dx.doi.org/10.1016/j.bbi.2011.04.004.

Green, M.J., Espie, C.A., Benzeval, M., 2014. Social class and gender patterning of insomnia symptoms and psychiatric distress: a 20-year prospective cohort study. BMC Psychiatry 14, 152. http://dx.doi.org/10.1186/1471-244X-14-152.

Heiser, P., Dickhaus, B., Schreiber, W., Clement, H.W., Hasse, C., Hennig, J., Remschmidt, H., Krieg, J.C., Wesemann, W., Opper, C., 2000. White blood cells and cortisol after sleep deprivation and recovery sleep in humans. Eur. Arch. Psychiatry Clin. Neurosci. 250, 16–23.

Irwin, M., 2002. Effects of sleep and sleep loss on immunity and cytokines. Brain, Behav., Immunity 16, 503–512.

Irwin, M., McClintick, J., Costlow, C., Fortner, M., White, J., Gillin, J.C., 1996. Partial night sleep deprivation reduces natural killer and cellular immune responses in humans. FASEB J. 10, 643–653.

Kerkhofs, M., Boudjeltia, K.Z., 2012. From total sleep deprivation to cardiovascular disease: a key role for the immune system? Sleep 35, 895–896. http://dx.doi.org/10.5665/sleep.1938.

Lange, T., Dimitrov, S., Fehm, H.L., Westermann, J., Born, J., 2006. Shift of monocyte function toward cellular immunity during sleep. Arch. Internal Med. 166, 1695–1700. http://dx.doi.org/10.1001/archinte.166.16.1695.

Lange, T., Dimitrov, S., Born, J., 2010. Effects of sleep and circadian rhythm on the human immune system. Ann. N. Y. Acad. Sci. 1193, 48–59. http://dx.doi.org/10.1111/j.1749-6632.2009.05300.x.

Lasselin, J., Capuron, L., 2014. Chronic low-grade inflammation in metabolic disorders: relevance for behavioral symptoms. Neuroimmunomodulation 21, 95–101. http://dx.doi.org/10.1159/000356535.

Lekander, M., Andreasson, A.N., Kecklund, G., Ekman, R., Ingre, M., Akerstedt, T., Axelsson, J., 2013. Subjective health perception in healthy young men changes in response to experimentally restricted sleep and subsequent recovery sleep. Brain, Behav., Immunity 34, 43–46. http://dx.doi.org/10.1016/j.bbi.2013.06.005.

Majde, J.A., Krueger, J.M., 2005. Links between the innate immune system and sleep. J. Allergy Clin. Immunol. 116, 1188–1198. http://dx.doi.org/10.1016/j.jaci.2005.08.005.

Manconi, M., Ulfberg, J., Berger, K., Ghorayeb, I., Wesstrom, J., Fulda, S., Allen, R.P., Pollmacher, T., 2012. When gender matters: restless legs syndrome. Report of the "RLS and woman" workshop endorsed by the European RLS Study Group. Sleep Med. Rev. 16, 297–307. http://dx.doi.org/10.1016/j.smrv.2011.08.006.

Meier-Ewert, H.K., Ridker, P.M., Rifai, N., Regan, M.M., Price, N.J., Dinges, D.F., Mullington, J.M., 2004. Effect of sleep loss on C-reactive protein, an inflammatory marker of cardiovascular risk. J. Am. College Cardiol. 43, 678–683. http://dx.doi.org/10.1016/j.jacc.2003.07.050.

Miller, A.H., Maletic, V., Raison, C.L., 2009. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. Biol. Psychiatry 65, 732–741. http://dx.doi.org/10.1016/j.biopsych.2008.11.029.

Mullington, J.M., Simpson, N.S., Meier-Ewert, H.K., Haack, M., 2010. Sleep loss and inflammation. Best Pract. Res.: Clin. Endocrinol. Metabol. 24, 775–784. http://dx.doi.org/10.1016/j.beem.2010.08.014.

Nishitani, N., Sakakibara, H., 2007. Subjective poor sleep and white blood cell count in male Japanese workers. Ind. Health 45, 296–300.

Roberts, R.E., Duong, H.T., 2014. The prospective association between sleep deprivation and depression among adolescents. Sleep 37, 239–244. http://dx.doi.org/10.5665/sleep.3388.

Shoelson, S.E., Herrero, L., Naaz, A., 2007. Obesity, inflammation, and insulin resistance. Gastroenterology 132, 2169–2180. http://dx.doi.org/10.1053/j.gastro.2007.03.059.

Spiegel, K., Tasali, E., Leproult, R., Van Cauter, E., 2009. Effects of poor and short sleep on glucose metabolism and obesity risk. Nat. Rev.: Endocrinol. 5, 253–261. http://dx.doi.org/10.1038/nrendo.2009.23.

Tsujimura, T., Matsuo, Y., Keyaki, T., Sakurada, K., Imanishi, J., 2009. Correlations of sleep disturbance with the immune system in type 2 diabetes mellitus. Diabetes Res. Clin. Pract. 85, 286–292. http://dx.doi.org/10.1016/j.diabres.2009.07.001.

Please cite this article in press as: Lasselin, J., et al. Effect of long-term sleep restriction and subsequent recovery sleep on the diurnal rhythms of white blood cell subpopulations. Brain Behav. Immun. (2014), http://dx.doi.org/10.1016/j.bbi.2014.10.004

van Leeuwen, W.M., Lehto, M., Karisola, P., Lindholm, H., Luukkonen, R., Sallinen, M., Harma, M., Porkka-Heiskanen, T., Alenius, H., 2009. Sleep restriction increases the risk of developing cardiovascular diseases by augmenting proinflammatory responses through IL-17 and CRP. PloS One 4, e4589. http://dx.doi.org/10.1371/journal.pone.0004589.

van Mark, A., Weiler, S.W., Schroder, M., Otto, A., Jauch-Chara, K., Groneberg, D.A., Spallek, M., Kessel, R., Kalsdorf, B., 2010. The impact of shift work induced chronic circadian disruption on IL-6 and TNF-alpha immune responses. J. Occup. Med. Toxicol. 5, 18. http://dx.doi.org/10.1186/1745-6673-5-18.

Vgontzas, A.N., Zoumakis, E., Bixler, E.O., Lin, H.M., Follett, H., Kales, A., Chrousos, G.P., 2004. Adverse effects of modest sleep restriction on sleepiness, performance, and inflammatory cytokines. J. Clin. Endocrinol. Metabol. 89, 2119–2126. http://dx.doi.org/10.1210/jc.2003-031562.

Please cite this article in press as: Lasselin, J., et al. Effect of long-term sleep restriction and subsequent recovery sleep on the diurnal rhythms of white blood cell subpopulations. Brain Behav. Immun. (2014), http://dx.doi.org/10.1016/j.bbi.2014.10.004